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Urbanisation Alters Fatty Acids in Stream Food Webs

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Title: Urbanization alters fatty acids in stream food webs

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Summary:

1. Fatty acids are essential to macroinvertebrate growth and reproduction and can indicate food web structure and nutritional quality of basal resources. However, broad scale examination of how watershed land cover and associated stressors affect the proportions of fatty acids in stream food webs are few. Our goals were to (1) document proportions of fatty acids among benthic periphyton and macroinvertebrate collector/gatherers, shredders, and predators and (2) examine if relationships between periphytic and macroinvertebrate fatty acids were altered due to the intensity of urban development in watersheds.
2. Proportions of the ≥ 20-C eicosapentaenoic acid (EPA 20:5ω3), arachidonic acid (ARA 20:4□6), and docosahexaenoic acid (22:6ω3) indicated a more periphyton rich diet of collector/gatherers when compared to shredders, which had significantly lower proportions of these fatty acids. Collector/gatherers likely were high quality sources of □3 and ≥ 20-C fatty acids for predators, which also had significantly greater EPA and ARA proportions than those in shredders. Linoleic (18:2□6) and α-linolenic acid (18:3ω3) comprised the greatest proportions of fatty acids in shredders, which likely indicated a diet dominated by leaf litter and associated hyphomycetes.
3. As watershed urbanization increased, proportions of total □3 fatty acids and EPA in periphyton increased and appeared to propagate through macroinvertebrate consumers and predators, given that proportions of these fatty acids also were significantly correlated with factors affected by watershed urbanization. The significant increase in total □3 fatty acids and EPA proportions within shredders indicated that periphyton growth, and their fatty acids, increased on leaf litter likely due to greater nutrient

concentrations associated with watershed <5% impervious cover. Proportions of total ω6 fatty acids in biota were not significantly correlated with factors associated with urban development, which could indicate that they were of sufficient abundance for consumers regardless of urban intensity or possible changes in their sources.

4. Our study provides an informative first step that identified notable differences in proportions of fatty acids among macroinvertebrates in urban streams and an increase in proportions of total ω3 fatty acids and EPA in periphyton, consumers, and predators as watershed urbanization increases. Identifying how fatty acid relationships within food webs change in response to watershed alterations and stressors could inform land use and management decisions by linking environmental changes to measures important to ecosystem outcomes.

Introduction

Identifying how human activities in watersheds affect downstream habitats and ecological communities is important to informing the protection and management of stream ecosystems (Booth et al., 2016; Parr et al., 2016), particularly as human populations and development continue to increase and expand (Seto et al., 2011). Humans depend on water resources, but increasing watershed development and human population growth negatively affect these ecosystems and associated biota by altering geomorphology, hydrologic regimes, and water quality (Paul & Meyer, 2001; Walsh et al., 2005; Booth et al., 2016). High human population densities and greater impervious cover in developed watersheds increase nutrient concentrations via sewage overflow, impervious surface runoff, stream bank erosion, and reduced channel complexity and riparian habitat, which can lead to altered base flows in streams and flashy hydrology during storm events (Meyer et al., 2005; Smucker & Detenbeck, 2014; Bhaskar et al., 2016). These changes in water quality and habitat subsequently affect periphytic and benthic macroinvertebrate communities in urban streams (Roy et al., 2003; King et al., 2011; Smucker et al., 2013).

Degradation of stream habitat and increased nutrient concentrations decrease periphyton diversity and increase the abundance of disturbance-tolerant species (Murdock et al., 2004; Passy & Blanchet 2007). Benthic periphyton contribute the majority of primary production in streams, making them important in nutrient and organic matter cycling and for the dietary needs of higher trophic levels (Battin et al., 2003; Dodds et al., 2004; Costello et al., 2016). Changes in benthic periphyton assemblages can lead to increases in periphyton nitrogen and phosphorus content (O'Brien & Wehr, 2010), which can contribute to shifts in macroinvertebrate consumers toward taxa with faster growth rates and lower C:P ratios (Evans-White et al., 2009). Changes in

periphyton assemblages and nutrient concentrations also affect macroinvertebrate consumers due to differences in fatty acid production by periphyton (Torres-Ruiz et al., 2010; Hill et al., 2011; Guo et al., 2016a). While stream macroinvertebrates are directly affected by changes in stream habitat and water quality (Urban et al., 2006; Wallace & Biastoch, 2016; Walsh & Webb, 2016), they also can be affected by changes in the quality of their diets mediated through shifts in periphyton and microbial communities, even in detrital-based food webs (Danger et al., 2013; Guo et al., 2016a). Streams with an increasing amount of agricultural intensity in their watersheds had periphytic assemblages more dominated by motile diatoms and fewer chlorophyte taxa while streams without intense agriculture were characterized by more prostrate diatoms and a greater diversity of chlorophyte and chrysophyte algae (Whorley & Wehr 2016a). These changes in assemblage composition can result in significant differences in the diversity of nutritional compounds produced as well as overall quantities due to the interaction with anthropogenic nutrient additions.

The ecological importance of fatty acids is receiving increased recognition because of their critical biochemical roles in animals, their use in describing food webs, and their potential effects at the ecosystem-scale (Twining et al., 2016). In urban streams, altered light availability and elevated nutrients can change the fatty acid and stoichiometric content of basal resources, such as periphyton, for macroinvertebrate consumers (Hill et al., 2011; Cashman et al., 2013; Guo et al., 2016b). Benthic macroinvertebrates depend heavily on fatty acids from periphyton because of their inability to synthesize the long chain, polyunsaturated fatty acids required for growth, neurotransmission, and regulation of physiology, hormone production, and behavior important to reproduction (Stanley-Samuelson 1994; Ravet et al., 2003; Arts et al., 2009). As a result, the fatty acid content of stream organisms can indicate how anthropogenic stressors affect

nutritional quality of periphyton and subsequent food webs (Torres-Ruiz et al., 2007; Larson et al., 2013; Boëchat et al., 2014). Characterizing relationships between land use and fatty acid content could provide information regarding how development, management, and restoration in watersheds affect streams. However, broad surveys examining these relationships are limited to only a few examples for seston in rivers (Larson et al., 2013; Boëchat et al., 2014).

In this study, we collected benthic periphyton, primary consumer macroinvertebrates, and predatory macroinvertebrates from second- to fourth-order streams spanning highly forested to suburban and highly urban watersheds. Our aim was to document the effects of urban development intensity in watersheds on fatty acid content of benthic periphyton and macroinvertebrates and to examine if relationships between periphyton and macroinvertebrate fatty acids content changed as a result. We hypothesized that (1) fatty acid (FA) content would differ among macroinvertebrate functional feeding groups due to dissimilarity of diets and (2) FA content within functional feeding groups would be reduced with increasing urbanization intensities due to increased stressor severity (impervious surfaces and chloride concentration) and nutrient enrichment.

Materials and Methods

Study streams within the Narragansett Bay watershed

We used a random sampling design to select 74 stream sites along a gradient of watershed development intensity (Smucker et al., 2016) in the Narragansett Bay watershed, in northeastern United States (Fig. 1). This 4421 km^2 watershed is one of the most densely populated in the United States with 380 people km^{-2} and approximately 35% developed land cover (US EPA 2007). For a complete characterization of the watershed, see site description in Smucker et al., 2016. The watershed of each sampled stream was delineated using NHDPlus

Basin Delineator Software (www.horizon-systems.com) and checked for accuracy using U.S. Geological Survey 7.5-min quadrangles (1:24,000). Land cover in watersheds was generated from photo-interpreted aerial imagery with 0.6 m and 0.5 m resolution for Rhode Island and Massachusetts, respectively (www.edc.uri.edu/rigis; www.mass.gov/anf/research-and-tech/itserv-and-support/application-serv/office-of-geographic-information-massgis). All land cover characterizations were conducted using ArcGIS 9.3 (Environmental Systems Research Institute,

Redlands, California U.S.A.). Watersheds of sampled streams ranged from 15.2–91% forest, 1.5–39.5% Impervious Cover (IC), and 3–1519 people km^{-2}.

Sample collection in streams

Streams were sampled between late July and early October 2012 during typical base flow conditions. At each stream, water for background chemical analysis was collected in an acidwashed (10% HCl) 1-L polypropylene container. Benthic periphyton samples were collected from each stream by selecting six cobbles, approximately 10–15 cm in diameter, evenly distributed within a 50-m reach. Attached benthic periphyton were removed from all above streambed surfaces of the selected cobbles using a firm-bristled brush (Stevenson & Bahls, 1999). We recognize that biofilms contain non-algal microbes and detritus, but for purposes of this study, we refer to the collected material as benthic periphyton because of the importance of algal-derived FAs. The pooled volume of periphyton material was measured and stored in acidwashed (10% HCl) polypropylene containers. Benthic macroinvertebrates were qualitatively sampled by conducting at least five two-minute kick-net collections. Additional macroinvertebrates were collected from periphyton scrapings, a visual survey of an additional six cobbles, and from leaves and woody debris when observed. Stream water, periphyton, and

macroinvertebrate samples were stored on ice in the dark until being processed within 24 h after collection. Macroinvertebrates were sorted by family and along with periphyton samples were frozen under N_2 gas until analyzed for FA.

Laboratory analyses

Stream water was filtered through a 0.2-µm syringe filter and analyzed for solublereactive phosphorus (SRP), nitrate/nitrite (NO_3^-/NO_2^-), ammonium (NH_4^+), dissolved organic carbon (DOC), and chloride (Cl^-). Unfiltered samples were persulfate digested for analysis of total nitrogen (TN) and total phosphorus (TP). Nutrient concentrations were determined using a Lachat flow-injection analyzer (Lachat Instruments, Milwaukee, WI, U.S.A.). DOC was determined by UV-promoted, persulfate oxidation on an organic carbon analyzer (Tekmar–Dohrmann Model Phoenix 8000, Teledyne Tekmar, Mason, OH, U.S.A.). Chloride was measured using ion chromatography (Dionex DX 600, Dionex Corporation, Sunnyvale, CA, U.S.A.; APHA 1998; US EPA 1987)

Macroinvertebrates were identified to family taxonomic level and categorized into functional feeding groups (FFG): collector/gatherers, shredders, and predators (Table 1; Cummins & Klug, 1979). Scrapers were rarely observed in general, and when present at sites they had low abundance and were unavailable for FA analysis after being used for other aspects of this research. Macroinvertebrates were blotted dry and periphyton samples were filtered onto ashed GF/F filters (GE/Whatman, Buckinghamshire, UK). All samples were stored at -20° C in chloroform-washed borosilicate test tubes after being flushed with N_2 gas for FA analysis.

To extract FAs, periphyton and macroinvertebrate samples were homogenized using a tissue tearor and extracted in chloroform:methanol (2:1). Extracted FAs were methylated using BF_3, and transferred to a hexane solvent (after Parrish, 1999; Torres-Ruiz et al., 2007; Whorley

& Wehr, 2016a). Nonadecanoic acid (19:0) was used as an internal standard to test methylation efficiency and to assess consistency among sample runs along with blank hexane samples. Samples were analyzed and quantified using a Shimadzu GC-2014 fitted with a capillary column (Omegawax320, 30 m x 0.32 mm x 0.25 □m film thickness; Supelco®, Bellefonte, PA, USA). The temperature program has an initial injection into a splitless inlet at a temperature of 100° C, followed by 1-hour ramping to 260° C in increments of 10° C/10 min with helium as the carrier gas to an FID detector. A standard dilution series for analysis and standard curves of the FA compounds was made from a Supelco® 37 component FAME mix. Quantification focused on compounds with ≥18C, because many biologically important fatty acids are derived from ≥18C base molecules, and while algal and non-algal sources of 18C FAs exist, algae are primarily responsible for elongating and desaturating lipids beyond 18C (Stanley-Samuelson, 1994; Olsen, 1999; Torres-Ruiz et al., 2010). Detection limits of the GC were set to identify peaks that correspond to an average concentration minimum of 0.011 – 0.024 mg/m^2 (for benthic periphyton samples) depending on the size of the molecule, although well-cleaned samples can yield lower detectable values.

Statistical Analysis

We examined the content of 18 FAs in periphyton and macroinvertebrates with a focus on the following: proportions of total ≥18C FAs of □□3, □□6, □other, and the most commonly reported and important essential FAs. The essential FA examined were α-linolenic acid (ALA 18:3ω3), eicosapentaenoic acid (EPA 20:5ω3), docosahexaenoic acid (DHA 22:6ω3), linoleic acid (LIN 18:2□6), and arachidonic acid (ARA 20:4□6), as macroinvertebrates are most likely (or rarely with very limited ability) to desaturate and elongate from shorter-chained fatty acids. We quantified data as proportions of FAs because they represent changes of individual FAs

relative to the others. Non-parametric Kruskal-Wallis ANOVAs were used to determine if proportions of FA content were significantly different among periphyton and FFGs regardless of watershed % impervious cover; Dunn's tests were used to examine significant differences of medians. We used site means of each FFG for examining FA relationships with environmental variables. Ternary plots were used to compare distributions of periphyton and macroinvertebrates based on their proportions of □□3, □□6, and □other fatty acids. To aid with interpretation of these ternary plots, we used non-parametric Mann-Whitney U tests to examine if proportions of these three major groups of FAs in periphyton and FFGs differed significantly between the least disturbed sites with < 5% watershed impervious cover and more urban sites with > 5% watershed impervious cover (Sokal & Rohlf, 1995). This criterion is frequently reported as a level beyond which substantial changes in biota occur (Utz et al., 2009; King et al. 2011; Smucker et al., 2013). Non-parametric Spearman's Rank correlations were used to examine relationships of periphyton and macroinvertebrate FA content with stream nitrate and chloride concentrations, watershed population density (people km^{-2}), and percent impervious cover. The *a priori* α level for all tests was set at $\alpha = 0.05$. All statistical analyses were conducted using SYSTAT 13 and SigmaPlot 13 (Systat Software, Inc., Chicago, IL, USA).

Results

Summary of fatty acids in stream periphyton and macroinvertebrates

Of the 74 sites sampled, both periphyton and macroinvertebrates were able to be collected from 52 sites, with 15 sites having collector/gatherers, 25 with shredders, and 38 with predators (Table 1). Darner dragonfly larvae (Aeshnidae) and dobsonfly larvae (Corydalidae) were the most common predators, larval crane flies (Tipulidae) and case-building caddisflies (Limnephilidae) were the most common shredders, and the most common collector/gatherers

were net-spinning caddisflies (Hydropsychidae) and small crayfish (Cambaridae). other FAs, typically dominated by stearic acid (18:0) and oleic acid (18:1 9), comprised the greatest proportions of FAs in periphyton, shredders, and predators (Table 2), with 6 being intermediate and 3 being least abundant; collectors had approximately equal proportions of these three major groups of FAs (Fig. 2).

Major structural groups of FA compounds were significantly different among organisms analyzed (Fig. 2). Proportions of 3 FAs in periphyton (mean ± SE, 0.13 ± 0.01) were significantly lower than those in macroinvertebrate FFGs (H_3 = 60.747, P < 0.001), which were higher and similar to each other (shredders 0.26 ± 0.03; predators 0.27 ± 0.02; collector/gatherers 0.31 ± 0.03). The proportions of 6 FAs among periphyton and FFGs were not significantly different (H_3 = 5.055, P = 0.168; periphyton 0.30 ± 0.01; collector/gatherers 0.35 ± 0.03; shredders 0.36 ±0.04; predators 0.33 ± 0.02). Proportions of other FAs in periphyton (0.58 ± 0.01) were also significantly greater than those in macroinvertebrate FFGs (H_3 = 68.637, P < 0.001), which were similar to each other (collector/gatherers 0.35 ± 0.03; shredders 0.38 ± 0.04; predators 0.40 ± 0.02). Macroinvertebrate FFGs had similar variation in the three major groups of FAs (Figs. 3a).

Of the 18 FA compounds quantified in this study (Table 2), important essential 3 FA compounds included -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Figs. 4a-c). While quantified proportions of all three compounds were significantly different among periphyton and macroinvertebrate FFGs (H_3 > 21.0, P < 0.001), periphyton contained the lowest proportions of all three compounds. Proportions of ALA were the greatest and most variable in shredders (0.18 ± 0.03). Predators had the greatest proportions of EPA (0.15

± 0.01), while collector/gatherers had the greatest proportion of DHA (0.013 ± 0.003). The important essential □6 FA compounds linoleic acid (LIN; $H_3 = 23.966$, $P < 0.001$) and arachidonic acid (ARA; $H_3 = 42.367$, $P < 0.001$) and followed similar patterns of significance between periphyton and macroinvertebrate FFGs (Figs. 4d-e). Shredders (0.26 ± 0.04) had the greatest proportion of LIN while collector/gatherers had the greatest proportion of ARA (0.13 ± 0.02).

Fatty acid relationships with urban stressors

Land cover characteristics (previously described in Smucker et al., 2016) and stream water chemistry varied greatly among streams (Table 3). The proportions of □□3 FAs in all macroinvertebrates, regardless of feeding group, were greater at urban sites with > 5% watershed impervious cover than in those at least disturbed sites with < 5% impervious cover (Fig. 3b; Mann-Whitney U test, $P < 0.05$), as were the proportions of □□3 of collector/gatherers and predators (Mann-Whitney U test, $P < 0.05$; shredders $P = 0.07$). The proportions of collector/gatherer EPA and predator EPA were greater in urban sites than in least disturbed sites (Mann-Whitney U test, $P < 0.05$). Proportions of □□6 and □other in FFGs were not significantly different between least disturbed and urban sites. Proportions of major groups of FAs in periphyton did not significantly differ between urban and least disturbed sites (Fig. 3c).

The proportion of □□3 among macroinvertebrate FFGs increased with increasing impervious cover in watersheds, chloride, and nitrate concentrations (Fig. 5, Table 4). Proportions of □□3 in shredders and predators were most strongly correlated with increasing impervious cover ($r_s = 0.42$, 0.39, respectively; $P < 0.05$), nitrate concentrations ($r_s = 0.44$, 0.32, respectively; $P < 0.05$), and chloride concentrations ($r_s = 0.55$, 0.39, respectively; $p < 0.05$). The proportion of □□3 in periphyton increased with higher population densities in watersheds and

chloride concentrations (r_s = 0.24, 0.26, respectively; $P < 0.05$). Interestingly, proportions of □□□ FA in periphyton and macroinvertebrate FFGs were not correlated with variables associated with urbanization. The proportions of □other FAs in periphyton, shredders, and predators decreased with greater watershed percent impervious cover (r_s = -0.29, -0.41, and -0.32, respectively; $P < 0.05$). The proportions of □other FAs in periphyton and collectors decreased with higher population densities in watersheds (r_s = -0.37, -0.60, respectively; $P < 0.05$).

The proportion of EPA in periphyton increased with greater watershed percent impervious cover, concentrations of chloride, and population density ($r_s > 0.30$, $P < 0.01$; see Table 4). Collectors had few significant relationships, with only ALA increasing along with watershed percent impervious cover (r_s = 0.60, $P < 0.05$). Shredder EPA proportions were positively associated with concentrations of nitrate, chloride, and impervious cover ($r_s > 0.35$, $P < 0.05$), whereas their proportions of DHA negatively associated with watershed impervious cover and population density ($r_s < -0.40$, $P < 0.05$). The proportion of EPA in predators increased with greater watershed percent impervious cover (r_s = 0.51, $P < 0.01$), chloride (r_s = 0.49, $P < 0.01$), and population density (r_s = 0.37, $P < 0.05$).

Discussion

Comparisons of fatty acids among periphyton and macroinvertebrates

Quantifying the proportions of fatty acids among algae and FFGs can provide information on the structure and nutritional qualities of food webs, but studies including an examination of predators and how environmental changes affect FAs of multiple consumers in natural ecosystems have been uncommon, particularly at large scales (Twining et al., 2016; Guo et al., 2016a). In our study of a large watershed, macroinvertebrates had greater proportions of □□3 FAs and lower proportions of □other FAs than those in periphyton, whereas their

proportions of □□6 were similar. These patterns likely resulted from macroinvertebrates having the ability to selectively retain periphyton derived FAs even if food sources have low amounts of them, especially ALA, EPA, and ARA (Brett et al., 2017; Crenier et al., 2017; Guo et al., 2017). These compounds also indicate higher food quality than shorter-chained or saturated FAs (Torres-Ruiz et al., 2007; Guo et al., 2016a). Although proportions of □□3, □□6, and □other fatty acid groups did not significantly differ among shredders, collector/gatherers, and predators, their proportions of the five key essential FAs did. These differences likely resulted from dissimilar diets among feeding groups, given that fatty acid content and proportions differ among basal resources in streams, and most macroinvertebrates are unlikely, or are at least greatly limited in their ability, to alter their content of these FAs via desaturation and elongation (Torres-Ruiz et al., 2007; Torres-Ruiz et al., 2010; Guo et al., 2016a; Guo et al. 2017).

Of the five essential FAs, LIN had, on average, either the greatest or second greatest proportion in periphyton and across all macroinvertebrate feeding groups. EPA was measured in the greatest proportion in collector/gatherers, ALA was the second greatest proportion in shredders, and EPA was the second greatest proportion in predators. Collector/gatherers likely consume a variety of basal resources, but their proportions of longer-chained FAs, especially EPA, ARA, and DHA, were significantly greater than those in shredders, which suggests that they benefited from a more periphyton-rich diet (Taipale et al., 2013; Whorley & Wehr, 2018). These results also indicated that diatoms likely comprised a substantial portion of their diets given that diatoms are the most common stream alga group which produces EPA, DHA, and ARA, whereas chlorophytes and cyanobacteria produce greater amounts of shorter-chained ALA and LIN (Harwood & Guschina, 2009; Taipale et al., 2013; Galloway & Winder, 2015, Richoux et al., 2018). Collector/gatherers (excluding Cambaridae) likely were high quality sources of

EPA and ARA for predators. However, shredders possibly were the dominant prey in predator diets, given that proportions of EPA and ARA in predators were greater than those in shredders and less than those in collector/gatherers. These essential FAs have been reported to increase in secondary consumers (Persson & Vrede, 2006; Guo et al., 2016a); this is because proportions in predators are expected to be greater than those in collector/gatherers, if collectors/gatherers were their dominant prey.

A study of stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ in this same system provide results complimentary to the FAs in the present study, indicating a dominance of detrital pathways in these stream food webs across the urban gradient (Smucker et al., 2018). Although not quantified, most sites observationally had an abundance of riparian trees and in-stream leaf litter. Many terrestrial plants have undetectable amounts of highly unsaturated FAs and are prolific producers of ALA and LIN (Simopoulos, 1999; Mills et al., 2001), which had the greatest proportions in shredders. The high proportions of LIN, and even stearic acid (18:0) and oleic acid (18:1ω9), in periphyton and all feeding groups also indicated that detrital food sources likely were quite abundant, even as a component of periphyton, which can be comprised of > 30% allochthonous material (Rasmussen, 2010). In addition, fungi, especially hyphomycetes, can enhance the nutritional quality of detritus and likely were important contributors to the high proportions of LIN, ALA, oleic acid, and stearic acid (18-C FAs) in shredders and even in other FFGs (Arce-Funck et al., 2015; Vonk et al., 2016; Taube et al., 2018). Quantification of major periphyton groups could provide additional insights in the future (e.g., Whorley & Wehr, 2016a), given that increased abundances of chlorophyte and cyanobacteria taxa also could contribute to greater ωω6 FAs, LIN, and the ω3 ALA (Hill et al., 2011; Galloway & Winder, 2015; Guo et al.,

2016a). However, macroscopic filaments or mats of these types of algae visually appeared to be uncommon in periphyton at our sites.

Reponses of fatty acids to urban stressors

Proportions of □□6 FAs, LIN, and the □3 ALA were not significantly correlated with factors affected by urbanization, which could indicate that they were of sufficient abundance for consumers regardless of urban intensity or possible changes in their sources. Even given the significant differences in proportions of essential FAs among FFGs, proportions of □□3 FAs and EPA in periphyton, shredders, and predators increased with factors associated with greater amounts of urbanization, particularly watershed impervious cover, nitrate, chloride, and human population density (Walsh et al., 2005; Booth et al., 2016). Similar increases in □3 FAs of sestonic algae in large rivers affected by urban development have been observed (Boëchat et al., 2014; Larson et al., 2013), but our study is the first report for benthic periphyton, along with macroinvertebrate consumers and predators, from a large survey of low-order streams affected by a gradient of watershed development.

Although not correlated with nutrients in our study, increases in proportions of □□3 FAs and EPA in periphyton could have been associated with increased nutrients in more urban watersheds, given their well-documented mechanistic links to fatty acid production (Dalu et al., 2016; Guo et al., 2016b; Whorley & Wehr, 2018), though high levels of nutrients also could lead to reduced proportions of ≥ 20-C FAs (Cashman et al., 2013). Within agricultural systems, increases in available stream nutrients resulted in biofilms achieving greater concentrations of important fatty acid compounds, despite agricultural streams exhibiting decreased taxonomic diversity and more eutrophic indicative taxa (Whorley & Wehr, 2016a, Whorley & Wehr 2018). Several other studies have observed increases in FA availability in algal seston due to increasing

nutrient availability from anthropogenic activity (Larson et al., 2013; Boëchat et al., 2014). Stable isotope ratios of $\delta^{15}N$ in periphyton and macroinvertebrates from these sites indicated that human-related sources of nutrients were increasingly assimilated into biota as watershed urbanization became greater (Smucker et al., 2018). The strong correlations of ω3 FAs and EPA proportions with chloride concentrations could indicate nutrient effects as well, because chloride is a conservative tracer of water delivered to streams from sources affected by human activities, and is less affected than nutrients by biological uptake and transformation. Canopy cover by riparian trees, which was qualitatively abundant at most sites, may have further promoted higher proportions of periphyton EPA and ω3 FAs by reducing oxidative damage to their carbon double bonds and by reducing the amount of surplus carbon stored as saturated, monounsaturated, and shorter 18-C FAs under high light conditions (Hill et al., 2011; Cashman et al., 2013; Twining et al., 2016).

In our study watershed, as urbanization increased, proportions of ω3 FAs and EPA in basal resources appeared to propagate through macroinvertebrate consumers and predators; this occurred at the same time that proportions of these FAs were also significantly correlated with factors affected by watershed urbanization. Collector/gatherers were an exception to this pattern, though their marginally non-significant correlations were likely due in part to their smaller sample size. The significant increase in ω3 FAs and EPA proportions in shredders indicated that periphyton growth, and their FAs, increased on leaf litter and/or transported matter, as watershed urbanization increased (Guo et al., 2016c). An increase in proportions of ω3 FAs and EPA of periphyton and macroinvertebrates may seem like beneficial responses to urbanization, but these possibly could be outweighed by the negative and well-documented effects of altered

habitat, hydrology, and other stressors on the biomass and diversity of periphyton and macroinvertebrate communities (Roy et al., 2003; Moore & Palmer, 2005; Smucker & Detenbeck, 2014; Hoyle et al., 2017; Pearson et al., 2017). In addition, sensitive periphyton and macroinvertebrate taxa that thrive in minimally impacted systems are replaced by those with faster growth rates or higher P content under greater nutrient availability and by those more tolerant to altered habitat and deteriorated water quality (Stevenson et al., 2008; King et al., 2011; Tsoi et al., 2011; Morse et al., 2012). Further research could determine if increased fatty acid availability along urban gradients contributes to community turnover in a manner similar to that of increased nutrient availability, especially given the importance of FAs in growth, behavior, emergence, and reproduction of macroinvertebrates.

Conclusions

Our watershed-scale approach was useful for examining differences in fatty acid profiles within a portion of stream food webs and how they changed in response to watershed urbanization. However, future efforts would benefit from combining fatty acid profiles with quantitative measures of biomass, densities of individuals, and finer taxonomic resolution of periphyton and macroinvertebrates, along with additional characterization of basal resources. We collected the most common families of macroinvertebrates in the watershed (Gould, 1993), but some intra-family differences in feeding strategies among species and changes in species' diets during larval growth can exist. Additionally, consumers may exhibit more selective small-scale feeding on biofilm material and leaf-litter than previously considered. Taxa-specific fatty acid profiles are particularly sparse for streams, and future assessment of food web changes could benefit from evaluating temporal and developmental variation, as well as among and within species variability in their dietary needs, uptake, and content of FAs, which could provide

insights on mechanistic links to macroinvertebrate community structure (Cavaletto & Gardner, 1999; Torres-Ruiz et al., 2007; Whorley & Wehr, 2016b).

Even given the likely sources of variability, our study provides an informative first step that identified (1) notable differences in proportions of FAs among FFGs in urban streams and (2) an increase in proportions of □□3 FAs and EPA in periphyton, consumers, and predators as watershed urbanization became greater. These findings provide insights into the dietary, biochemical, and nutritional changes of biota and into potential trophic relationships. Given the importance of FAs to macroinvertebrate growth and reproduction, to higher trophic levels, and to terrestrial ecosystems (Twining et al. 2016), identifying how fatty acid relationships within food webs change in response to watershed alterations and stressors could inform land use and management decisions by linking environmental changes to measures important to ecosystem outcomes.

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680 **Table 1:** Mean proportions (±SE) of main structural fatty acid groups and counts for each family

681 of benthic invertebrate included in the analysis. Where no standard error is indicated, there was

682 only one organism. Each Family (*Order) is listed by its functional feeding group (FFG)

and for 683 each impervious cover (% IC) category.

684

		Mean Proportion FA (±SE)			Number of sites	
		□□3	□□6	□other	<5% IC	≥ 5% IC
	Asellota*	0.181 (n/a)	0.477 (n/a)	0.343 (n/a)		1
FFG	Family					
Collector/ Gatherers	Cambaridae	0.277 (0.041)	0.269 (0.019)	0.454 (0.045)	1	5
	Gammaridea	0.346 (0.035)	0.393 (0.062)	0.261 (0.050)		4
	Hydropsychidae	0.350 (0.089)	0.365 (0.071)	0.285 (0.024)	1	4
	Philopotamidae	0.556 (n/a)	0.188 (n/a)	0.256 (n/a)		1
Shredders	Limnephilidae	0.292 (0.048)	0.264 (0.045)	0.445 (0.068)	4	9
Predators	Aeshnidae	0.252 (0.028)	0.349 (0.030)	0.399 (0.030)	9	16
Tipulidae			0.228 (0.037)	0.458 (0.046)	0.314 (0.029)	3
			10			
	Calopterygidae	0.312 (0.072)	0.375 (0.084)	0.314 (0.012)		2
	Corydalidae	0.221 (0.018)	0.339 (0.038)	0.440 (0.041)	14	10
	Gomphidae	0.268 (n/a)	0.353 (n/a)	0.379 (n/a)		1
	Libellulidae	0.305 (0.025)	0.365 (0.020)	0.331 (0.034)		4
	Perlidae	0.322 (0.032)	0.280 (0.026)	0.399 (0.042)	6	4
	Rhyacophilidae	0.434 (0.168)	0.267 (0.095)	0.299 (0.073)		3

685

686

687 **Table 2:** Mean proportions (±SE) of each fatty acid compound quantified in this analysis.

688 Comparisons among trophic levels by Kruskal-Wallis ($df = 3$). Significance is $P < 0.05$. Essential

689 fatty acid compounds are indicated by their three-letter abbreviations and bold numbers. Totals

690 will not equal 1.0 due to averaging and rounding.

691

Lipid Formula	Periphyton	Collectors	Shredders	Predators	H (*P*)
18:0	0.143 (0.009)	0.122 (0.012)	0.123 (0.026)	0.158 (0.016)	11.806 (0.008)
18:1□9	0.346 (0.008)	0.136 (0.031)	0.140 (0.033)	0.175 (0.015)	71.885 (<0.001)
18:2□6 (LIN)	**0.117 (0.005)**	**0.174 (0.030)**	**0.259 (0.036)**	**0.192 (0.015)**	**23.966 (<0.001)**
18:3□6	0.141 (0.009)	0.038 (0.010)	0.061 (0.023)	0.045 (0.010)	76.289 (<0.001)
18:3□3 (ALA)	**0.071 (0.006)**	**0.079 (0.017)**	**0.184 (0.025)**	**0.103 (0.008)**	**21.780 (<0.001)**
20:0	0.010 (0.001)	0.015 (0.006)	0.027 (0.007)	0.016 (0.003)	2.998 (0.392)
20:1	0.010 (0.001)	0.015 (0.004)	0.033 (0.012)	0.007 (0.002)	24.426 (<0.001)
20:2	0.008 (0.001)	0.014 (0.003)	0.005 (0.002)	0.004 (0.0005)	17.298 (0.001)
20:3□6	0.001 (0.0002)	0.004 (0.001)	0.006 (0.002)	0.005 (0.001)	44.383 (<0.001)
20;4□6 (ARA)	**0.039 (0.004)**	**0.128 (0.017)**	**0.034 (0.007)**	**0.089 (0.007)**	**42.367 (<0.001)**
20:3□3	0.012 (0.003)	0.005 (0.001)	0.008 (0.003)	0.014 (0.004)	1.498 (0.683)
20:5□3 (EPA)	**0.039 (0.005)**	**0.214 (0.023)**	**0.068 (0.017)**	**0.150 (0.011)**	**77.867 (<0.001)**
22:0	0.015 (0.001)	0.026 (0.013)	0.033 (0.009)	0.030 (0.008)	18.719 (<0.001)
22:1□9	0.006 (0.001)	0.003 (0.001)	0.008 (0.005)	0.010 (0.005)	9.257 (0.026)
22:2	0.008 (0.001)	0.001 (0.001)	0.005 (0.004)	0.001 (0.0003)	32.027 (<0.001)
23:0	0.002 (0.001)	0.003 (0.001)	0.003 (0.001)	0.001 (0.001)	10.083 (0.018)
24:0	0.025 (0.002)	0.006 (0.002)	0.001 (0.0003)	0.0003 (0.0001)	109.565 (<0.001)
22:6□3 (DHA)	**0.003 (0.001)**	**0.013 (0.003)**	**0.001 (0.0002)**	**0.001 (0.0002)**	**28.207 (<0.001)**

692

693 **Table 3:** Summary of water chemistry and GIS land cover categories across all streams

694 surveyed.

	TC (ppb)	TP (ppb)	TN (ppb)	NO2/NO 3- (□g/L)	NH4+ (ppb)	PO4- (ppb)	Cl- (mg/L)	% IC	% Wetland	% Forest	% Agriculture
Min	1.583	1.148	100.442	7.599	7.300	3.310	3.212	0.0	0.0	15.201	0.0
Max	40.664	247.712	4,227.00	5,327.37	467.151	186.619	173.604	39.460	26.236	100.0	15.751
Mean (SE)	7.961 (0.601)	37.351 (3.273)	940.678 (50.390)	605.683 (56.681)	35.434 (3.542)	13.398 (1.178)	54.679 (2.870)	10.556 (0.592)	8.819 (0.566)	58.161 (1.397)	3.600 (0.253)
CV (%)	1.019	1.195	0.731	1.276	1.363	1.199	0.716	0.765	0.874	0.328	0.957

695

696 **Table 4** Spearman correlations of major FA groups' proportions in periphyton and invertebrate

697 feeding groups with urban-related environmental variables. □□6/total had no significant 698 correlations. Significant results ($P < 0.05$) are in bold.

	Impervious cover	Population density	Nitrate	Chloride
□□3/total EFA				
Periphyton	0.21 (0.08)	**0.24 (<0.05)**	-0.01 (0.91)	**0.26 (0.02)**
Collectors	0.48 (0.07)	0.46 (0.10)	0.30 (0.28)	0.48 (0.07)
Shredders	**0.42 (0.04)**	0.35 (0.09)	**0.44 (0.03)**	**0.55 (<0.01)**
Predators	**0.39 (0.01)**	0.28 (0.09)	**0.32 (0.05)**	**0.33 (0.04)**
□other/total EFA				
Periphyton	**-0.29 (0.01)**	**-0.37 (0.001)**	-0.08 (0.48)	-0.14 (0.24)
Collectors	-0.43 (0.11)	**-0.60 (0.02)**	-0.21 (0.46)	-0.28 (0.31)
Shredders	**-0.41 (0.04)**	-0.23 (0.27)	-0.18 (0.39)	-0.33 (0.11)
Predators	**-0.32 (0.05)**	-0.09 (0.59)	-0.22 (0.19)	-0.20 (0.23)

699

Figure 1 Map showing development intensities and the distribution of sampled streams in the 4421 km^2 Narraganset Bay Watershed located in northeastern United States of America. Natural

land cover includes forest, vegetated, and wetland land cover. NLCD = National Land Cover Database, RI = Rhode Island, MA = Massachusetts, CT = Connecticut.

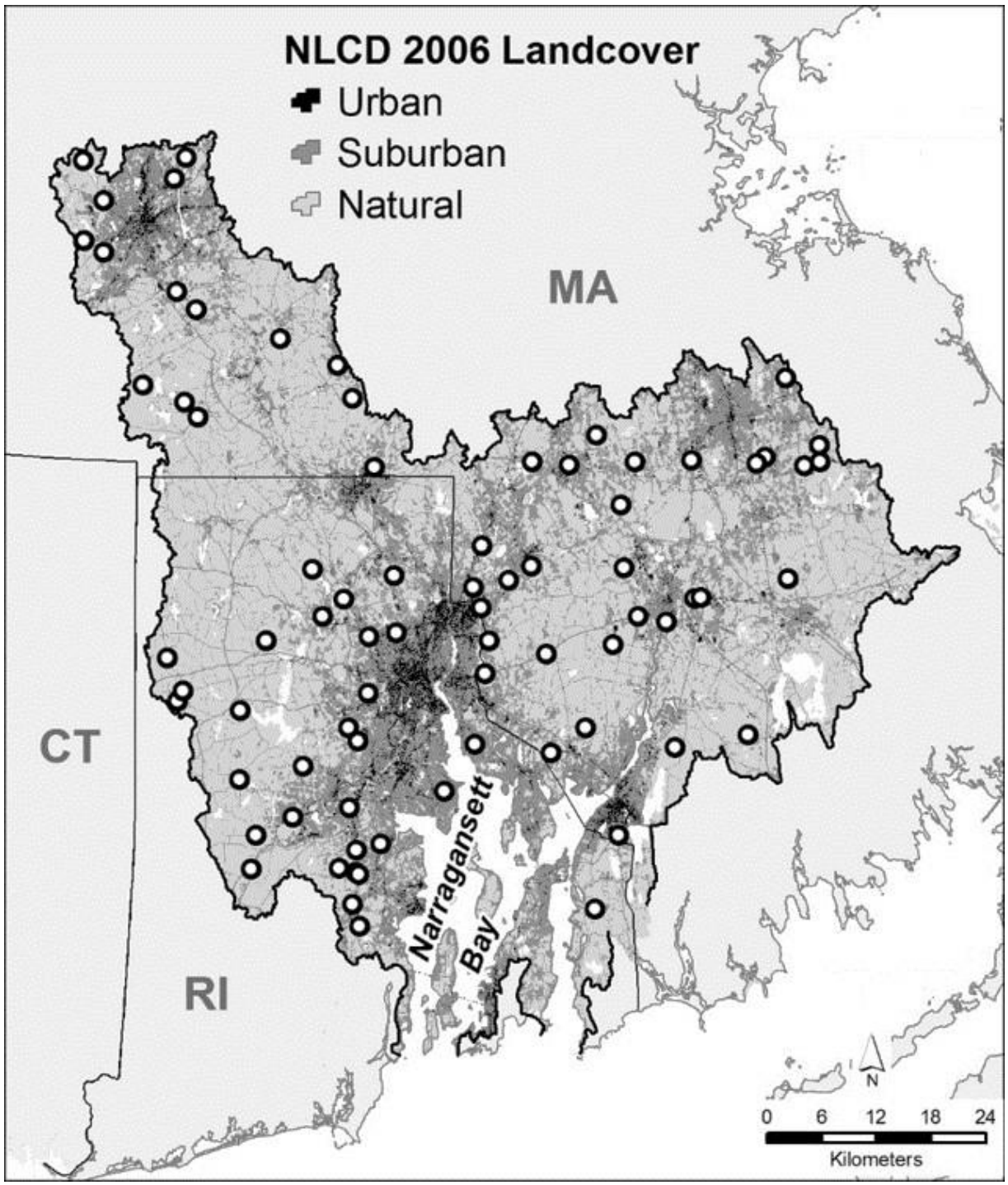


Figure 2 Box plots showing proportions of **(A)** □□3, **(B)** □□6, and **(C)** □other fatty acids of all periphyton and invertebrate collectors, shredders, and predators collected from all sites. Boxes are

interquartile ranges with lines showing medians and whiskers showing 10th and 90th percentiles. Boxes not sharing any letters are significantly different (Kruskal-Wallis ANOVA with Dunn's test for significant differences among medians).

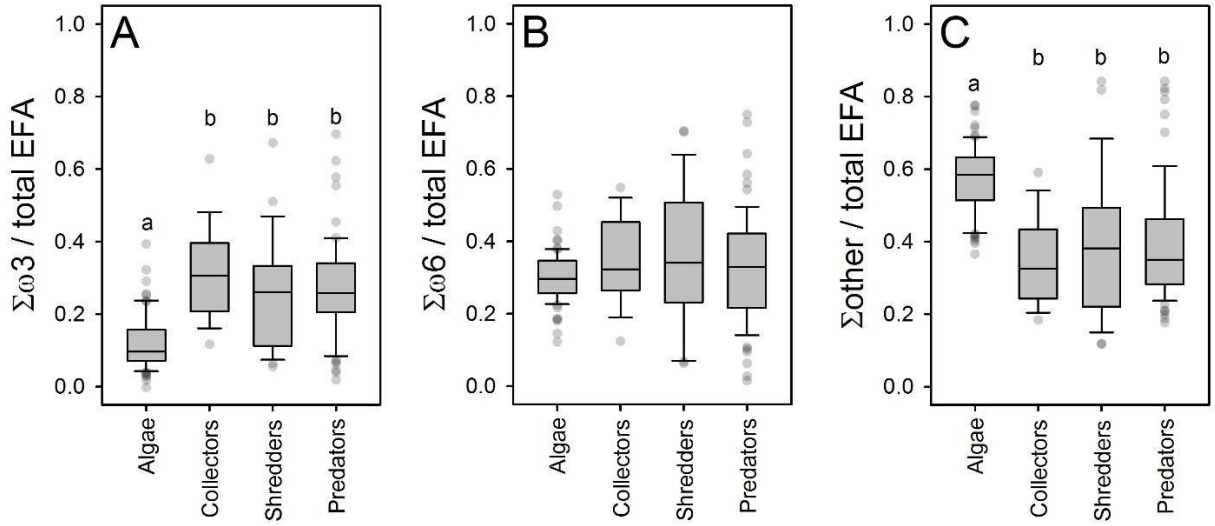


Figure 4 Box plots showing proportions of five essential fatty acids of all periphyton and invertebrate collectors, shredders, and predators collected from all sites including: **(A)** linolenic acid (ALA), **(B)** eicosapentaenoic acid (EPA), **(C)** docosahexaenoic acid (DHA; note different scale), **(D)** linoleic acid (LIN), and **(E)** arachidonic acid (ARA). Boxes are interquartile ranges with lines showing medians and whiskers showing 10th and 90th percentiles. Boxes not sharing any letters are significantly different (Kruskal-Wallis ANOVA with Dunn's test for significant differences among medians).

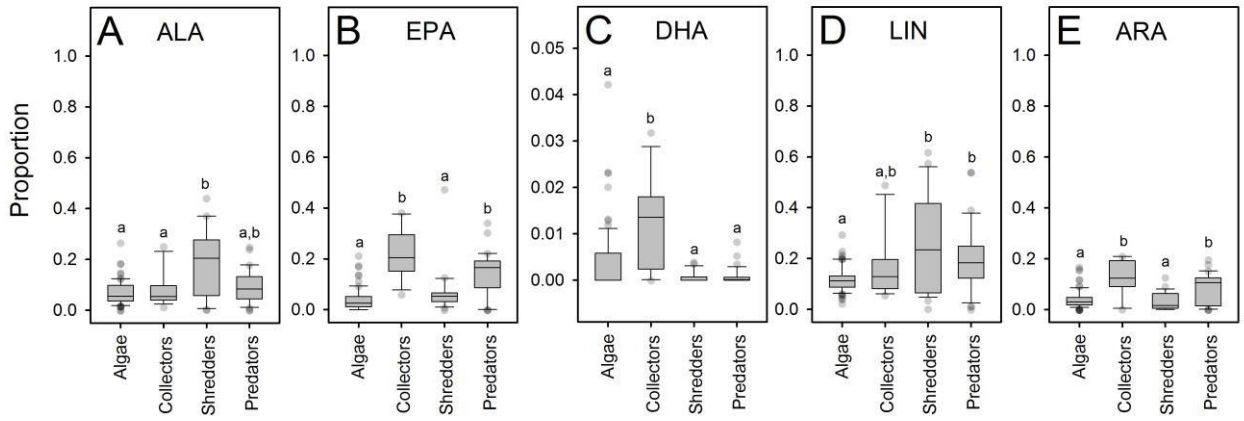
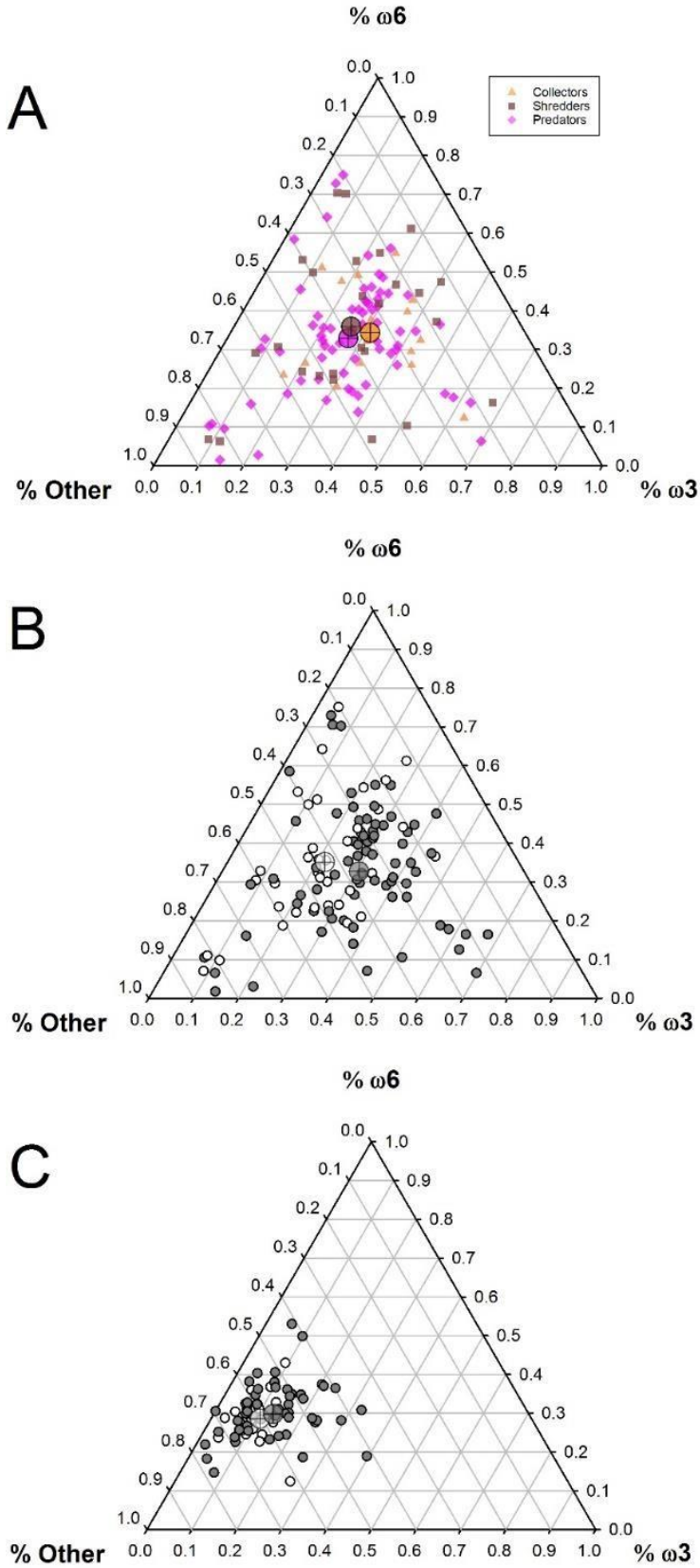
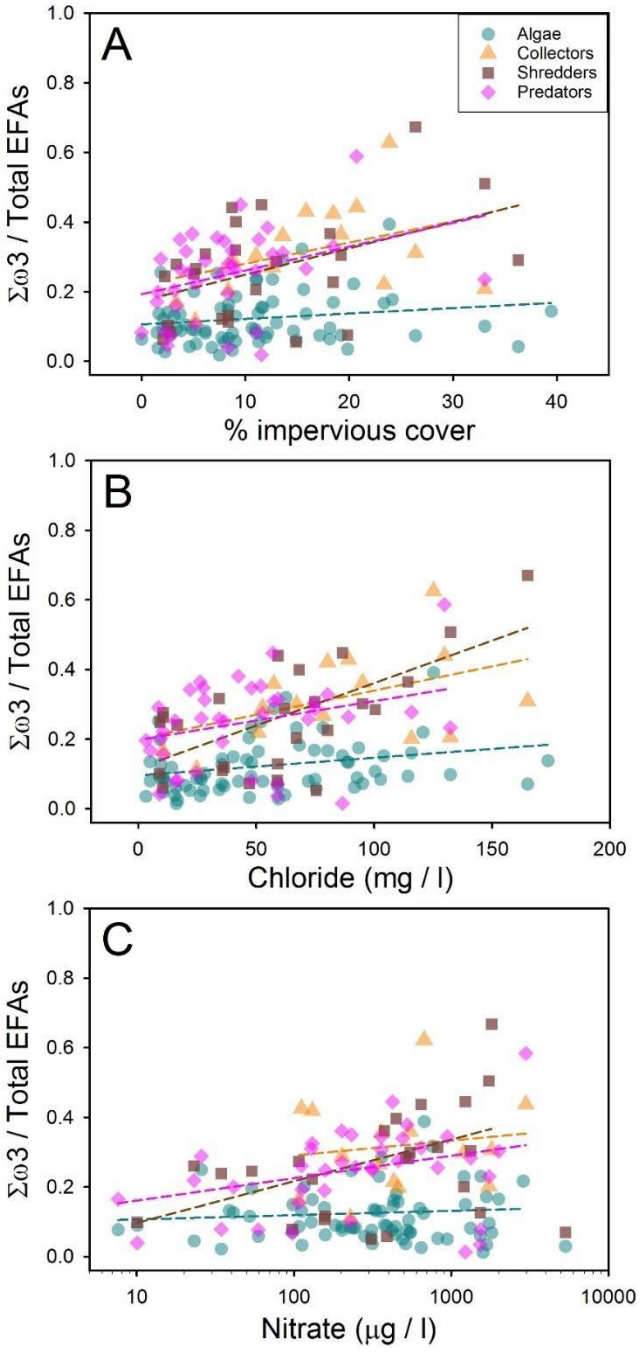

A ALA
B EPA
C DHA
D LIN
E ARA
Proportion
Algae
Collectors
Shredders
Predators

Figure 3 Ternary plots based on proportions of □□3, □□6, and □other fatty acids for **(A)** all macroinvertebrates collected in the study distinguished by functional feeding group and for **(B)** all macroinvertebrates and **(C)** periphyton distinguished by being collected from least disturbed streams with <5% watershed impervious cover (white) or from urban streams with >5% watershed impervious cover (gray). Larger crossed symbols indicate group mean values.



736

Figure 5 The □□3 / total fatty acids (FAs) ratios of periphyton and invertebrate functional feeding groups plotted against **(A)** percent watershed impervious cover, **(B)** chloride concentrations, and **(C)** nitrate concentrations. Lines are shown only to highlight relationships. See Table 4 for a breakdown of within-group correlations. An outlier of 5327 □g nitrate / L was excluded from fitted lines due to being nearly twice that of the next highest observed concentration.



760 Supplementary Table 1: Concentrations of all fatty acid compounds measured as mg/L for algae and mg/g for invertebrates. Reported

761 as the mean (± SE). Where a mean of "0.000" is reported indicates that none of that compound was measured. An SE of "n/a" 762 indicates that only one organism is included in that measurement.

	FFG	18:0	18:1□9	18LIN:2□ 6	18:3□6	18ALA:3□ 3	20:0	20:1□9	20:2	20:3□6	20ARA:4□6	20:3□3	20(EPA:5□)3	22:0	22:1□9	22:2
	Algae	0.091 (0.010)	0.268 (0.042)	0.100 (0.018)	0.076 (0.005)	0.081 (0.028)	0.007 (0.001)	0.008 (0.001)	0.005 (0.001)	0.002 (0.0004)	0.039 (0.008)	0.006 (0.001)	0.056 (0.015)	0.011 (0.001)	0.005 (0.001)	0.005 (0.0005)
Collector/Gatherers	**Asellota**	0.121 (n/a)	0.232 (n/a)	0.143 (n/a)	0.123 (n/a)	0.041 (n/a)	0.000 (n/a)	0.000 (n/a)	0.000 (n/a)	0.000 (n/a)	0.226 (n/a)	0.000 (n/a)	0.146 (n/a)	0.000 (n/a)	0.000 (n/a)	0.000 (n/a)
	Cambaridae	0.060 (0.012)	0.118 (0.031)	0.063 (0.018)	0.008 (0.005)	0.025 (0.004)	0.006 (0.001)	0.007 (0.001)	0.010 (0.003)	0.003 (0.001)	0.073 (0.019)	0.003 (0.001)	0.093 (0.018)	0.002 (0.001)	0.001 (0.001)	0.0001 (0.00007)
	Gammaridae	0.103 (0.042)	0.060 (0.019)	0.121 (0.055)	0.079 (0.034)	0.027 (0.009)	0.000 (n/a)	0.009 (0.05)	0.024 (0.009)	0.003 (0.003)	0.159 (0.043)	0.001 (0.001)	0.252 (0.057)	0.034 (0.034)	0.000 (n/a)	0.003 (0.003)
	Hydropsychidae	0.576 (0.446)	0.192 (0.150)	1.108 (0.452)	0.070 (0.042)	0.752 (0.540)	0.111 (0.053)	0.076 (0.036)	0.010 (0.007)	0.009 (0.005)	0.271 (0.235)	0.014 (0.011)	0.745 (0.497)	0.175 (0.107)	0.024 (0.022)	0.046 (0.044)
Shredders	**Philopotamidae**	0.149 (n/a)	0.064 (n/a)	0.066 (n/a)	0.017 (n/a)	0.178 (n/a)	0.011 (n/a)	0.003 (n/a)	0.003 (n/a)	0.003 (n/a)	0.083 (n/a)	0.003 (n/a)	0.320 (n/a)	0.000 (n/a)	0.000 (n/a)	0.000 (n/a)
	Limnephilidae	0.629 (0.377)	0.337 0.156 (0.101)	0.416 0.190 (0.153)	0.029 (0.013)	0.286 (0.057)	0.030 (0.016)	0.018 (0.008)	0.003 (0.001)	0.009 (0.004)	0.025 0.067 (0.009)	0.005 (0.003)	0.126 (0.056)	0.034 (0.017)	0.005 (0.003)	0.004 (0.002)
	Tipulidae	0.107	0.049	0.284	0.161 (0.111)	0.169 (0.068)	0.032	0.071 (0.050)	0.006 (0.003)	0.004 (0.002)	0.053	0.018 (0.015)	0.059 (0.018)	0.037 (0.016)	0.032 (0.031)	0.002 (0.001)
	Aeshnidae	0.135 (0.034)	(0.030)	(0.041)	0.070 (0.034)	0.061 (0.009)	0.012 (0.004)	0.011 (0.009)	0.004 (0.001)	0.006 (0.001)	(0.012)	0.015 (0.007)	0.116 (0.021)	0.038 (0.015)	0.018 (0.014)	0.002 (0.001)
	Calopterygidae	0.126 (0.090)	0.098 (0.095)	0.122 (0.067)	0.361 (0.308)	0.179 (0.005)	0.082 (0.065)	0.003 (0.001)	0.004 (0.0002)	0.000 (n/a)	0.077 (0.070)	0.002 (0.002)	0.266 (0.062)	0.139 (0.139)	0.000 (n/a)	0.000 (n/a)
	Corydalidae	0.208	0.139	0.114	0.019	0.048	0.010	0.008	0.003	0.004	0.047	0.018	0.079	0.016	0.006	0.002

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